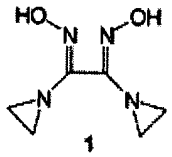
1-Aziridino-1-hydroxyiminomethyl Derivatives, Methods for Preparing Them, and Drugs Containing These Compounds

This application is a 371 of PCT/DE00/03441 09/22/2000.

This invention relates to 1-aziridino-1-hydroxyiminomethyl derivatives, methods for preparing them, and drugs containing these compounds.

Only one bis(aziridine oxime) of Formula 1 is known so far in the state of the art (Andrianov, V.G., Eremeev, A.V., Zh. Org. Khim (1991), 27, 112-16; Eremeev, A.V., Piskunova, I.P., Andrianov, V.G., Liepins, E., Khim. Geterotsikl. Soedin (1982), (4) 488-94; Musluoglu, E., Ahsen, V., J. Chem. Research (S) (1999), 142-143).



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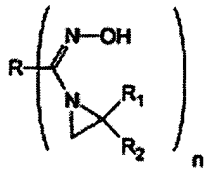
Nothing has yet been reported about the biological properties of this compound 1,1'-[1,2-bis(hydroxyimino)-1,2-ethanediyl]bisaziridine (1) or of its use as a drug.

Monoaziridine oximes that are used as herbicides, among others, are also known from DE-OS [Unexamined] 2,132,598. In the same way, aziridine oximes that are used to treat illnesses associated with the function of the chaperone system

are described in WO 97/16439. However, nowhere have bis-, tris-, or even tetraaziridine oximes been described.

The object of this invention is to make available new 1-aziridino-1-hydroxyiminomethyl derivatives with the general formula I



I

and a method for preparing them. Another object is to make available drugs that contain a compound with the general formula I.

In the general formula I, R stands for any organic residue that is able to bond covalently two aziridine oxime groups,

R_1 and R_2 independently of one another stand for a hydrogen atom or a $-CH_3$, $-C_2H_5$, -CN, -COOH, $-COOCH_3$, $-COOC_2H_5$, $-CONH_2$, or $-C_6H_5$ group, and n is the whole number 2.

It is preferred for R to be selected from a single bond, linear or branched, saturated or unsaturated alkanes or heteroalkanes with up to 6 carbon atoms

and with up to four hetero atoms, C_3-C_8 cycloalkanes that are optionally substituted with short-chain C_1-C_6 alkyl, C_1-C_6 alkoxy, nitro, amino, monosubstituted amino, and/or halogen groups, heterocyclic compounds with 3 to 6 ring atoms and up to four hetero atoms,
aromatic compounds with up to 8 ring atoms that are optionally substituted with cyano, hydroxy, short-chain C_1-C_6 alkyl, C_1-C_6 alkoxy, nitro, amino, monosubstituted amino, trihaloalkyl, and/or halogen groups, and heteroaryls with 3 to 7 ring atoms and up to four hetero atoms.

It is particularly preferred for the parent substance R to be selected from a single bond, methyl, ethane, ethene, ethyne, propane, isopropane, butane, isobutane, sec-butane, pentane, isopentane, neopentane, hexane, azine, cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, cyclooctane, pyrrole, pyrroline, pyrrolidine, imidazole, imidazoline, pyrazolidine, thiazole, thiazoline, thiazolidine, isothiazole, isothiazoline, isothiazolidine, benzothiazole, furan, dihydrofuran, tetrahydrofuran, benzofuran, thiophene, benzothiophene, oxazole, oxazoline, oxazolidine, benzoxazole, isoxazole, isoxazoline, isoxazolidine, piperidine, piperazine, pyrimidine, morpholine, dihydropyran, tetrahydropyran, pyridazine, benzene, furoxane, imidazole, imidazoline, imidazolidine, pyrazole, pyrazoline, pyrazolidine, pyridine and its N-oxide, dihydropyridine, pyrimidine, or pyrazine. It is clear that the hetero atoms are positioned at any points in the ring.

It is also preferred for R_1 and R_2 independently of one another to be hydrogen atoms or a $-CONH_2$ residue.

Very particularly preferred are

2,6-bis(1-aziridino-1-hydroxyiminomethyl)pyridine (6),

1,4-bis(1-aziridino-1-hydroxyiminomethyl)benzene (7),

1,4-di(α-2-carbamoylaziridino-α-hydroxyiminomethyl)benzene (8),

1,3-bis(1-aziridino-1-hydroxyiminomethyl)benzene (9),

1,3,5-tris(1-aziridino-1-hydroxyiminomethyl)benzene (10),

1,3-di(α-2-carbamoylaziridino-α-hydroxyiminomethyl)benzene (11),

2,6-di(α-2-carbamoylaziridino-α-hydroxyiminomethyl)pyridine (12),

3,5-bis(1-aziridino-1-hydroxyiminomethyl)pyridine (13),

2,5-bis(1-aziridino-1-hydroxyiminomethyl)pyridine ((14),

2,4-bis(1-aziridino-1-hydroxyiminomethyl)pyridine (15),

2,5-bis(1-aziridino-1-hydroxyiminomethyl)furan (16),

3,4-bis[(aziridinyl)-1-hydroxyiminomethyl]furoxane (17),

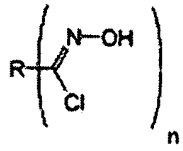
bis(2-methoxycarbonylaziridino)glyoxime (18),

bis(2-carbamoylaziridino)glyoxime (19),

2,2'-azinobis(1-aziridino-1-hydroxyiminomethyl)propane (20), and

2,2'-azinobis[1-(2-carbamoylaziridino)-1-hydroxyimino]propane (21).

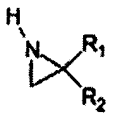
Another subject of this invention is a method for preparing 1-aziridino-1-hydroxyiminomethyl derivatives pursuant to the invention, by reacting a halogen



II

compound with the general formula II

wherein R and n have the meanings given above, in a known way with an aziridine derivative with the general formula III

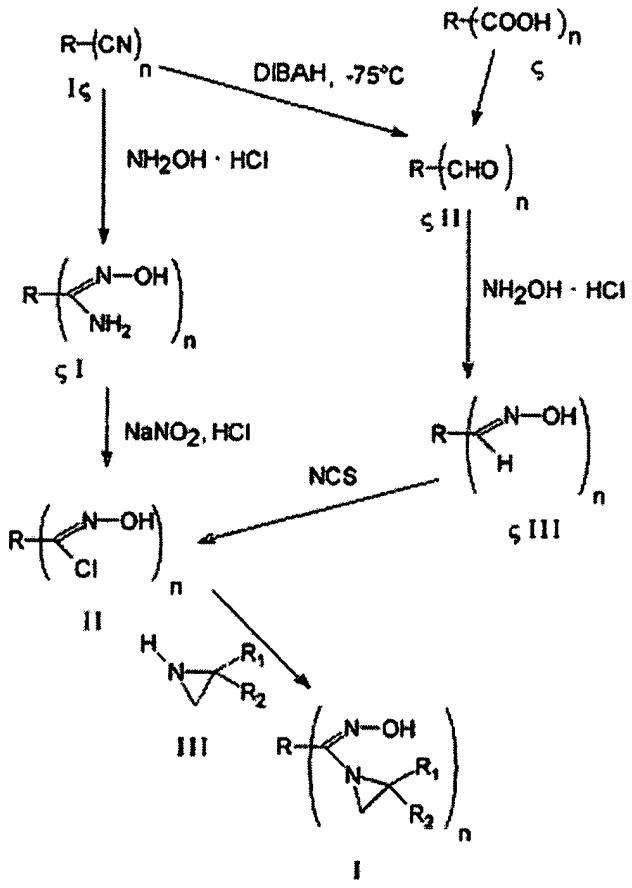


III

wherein R_1 and R_2 have the meanings given above.

The compounds of Formula I pursuant to the invention can be prepared by known methods according to the reaction diagram 1. To this end, nitriles with the general formula IV are converted to the carboxamide oximes with the general structure VI by reaction with hydroxylamine hydrochloride. By diazotization in hydrochloric acid medium, the chlorinated oximes of Structure II are obtained, which can then be converted to the compounds pursuant to the invention by reaction with aziridines of Formula III. Alternatively, as indicated in reaction diagram 1, the synthesis can be carried out starting with the carboxylic acids V by standard procedures described in the literature. The experimental method is indicated in the examples for the sequence IV → VI → II → I.

Reaction Diagram 1



Another subject of this invention is drugs characterized by containing a compound according to the general formula I.

Also a subject of this invention are drugs for oral, rectal, subcutaneous, intravenous, or intramuscular administration that contain a compound with the general formula I in addition to conventional vehicles and diluents.

Suitable dosage forms and their preparation are known for themselves and are described, for example in "Hagers Handbuch der pharmazeutischen Praxis" (*Hager's Manual of Pharmaceutical Practice*), Springer Verlag - Berlin - Heidelberg, 1991, Volume 2, pp. 622 ff.

The drugs of the invention are prepared by known methods with the customary solid or liquid vehicles or diluents and the pharmaceutical adjuvants customarily used for the desired method of administration, in suitable doses. The preferred preparations consist of a dosage form that is suitable for oral administration. Examples of such dosage forms are tablets, film-coated tablets, sugar-coated tablets, capsules, pills, powders, solutions or suspensions, or depot forms.

Of course parenteral preparations such as solutions for injection are also practical. Suppositories should also be mentioned as examples of preparations.

Appropriate tablets can be obtained, for example, by mixing the active ingredient with known adjuvants, for example, inert diluents such as dextrose, sugar, sorbitol, mannitol, polyvinylpyrrolidone, disintegrants such as corn starch or alginic acid, binders such as starch or gelatin, lubricants such as magnesium stearate or talc, and/or agents for producing a depot effect such as carboxypolymethylene, carboxymethylcellulose, cellulose acetate phthalate, or polyvinyl acetate. The tablets can also consist of several layers.

Correspondingly, sugar-coated tablets can be prepared by coating cores prepared similarly to the tablets with agents ordinarily used in coatings for sugar-coated tablets, for example polyvinylpyrrolidone or shellac, gum arabic, talc, titanium dioxide, or sugar. The shell of the sugar-coated tablet can also consist of several layers, for which the adjuvants mentioned above for tablets can be used.

Solutions or suspensions with the active ingredient pursuant to the invention can also contain, in addition, flavor-improving agents such as saccharin, cyclamate, or sugar, as well as flavorings such as vanillin or orange extract. The can also contain dispersants such as sodium carboxymethylcellulose or preservatives such as p-hydroxybenzoates. Capsules containing active ingredients can be prepared, for example, by encapsulating the active ingredient mixed with an inert carrier such as lactose or sorbitol in gelatin capsules.

Suitable suppositories can be prepared, for example, by mixing with vehicles intended for the purpose such as neutral fats or polyethylene glycol or their derivatives.

Of course transdermal therapeutic systems (TTSs) are also practical.

The compounds pursuant to the invention with the general formula I show antitumoral activity. The antitumoral activities of some compounds pursuant to the invention in the monolayer cytotoxicity test on selected cell lines are shown in

Table 1. The low susceptibility of fibroblasts and endothelial cells with the use of the compounds pursuant to the invention is surprising.

Another subject of this invention is therefore the use of the 1-aziridino-1-hydroxyiminomethyl derivatives with the general formula I for preparing drugs for the treatment of tumors or cancerous diseases.

However, the use of the 1-aziridino-1-hydroxyiminomethyl derivatives according to the general formula I for the treatment of tumors or of cancerous diseases is also a subject.

Another subject of this invention is the use of 1,1'-[1,2-bis(hydroxyimino)-1,2-ethanediyl]bisaziridine (1) to prepare drugs for the treatment of tumors or of cancerous diseases, and that of 1,1'-[1,2-bis(hydroxyimino)-1,2-ethanediyl]bisaziridine (1) for the treatment of tumors or of cancerous diseases.

Table 1:

Antitumoral activity of selected compounds pursuant to the invention

Substance IC_{50} [µg/ml]		1	6	14	7	9	10	16
Organ/cell line								
Colon	HT29	0.486	0.117	0.200	0.258	0.329	0.670	0.481
Stomach	GXF 251L	0.781	0.020	0.717	0.542	1.506	3.964	1.661
Lung	LXFL 529	0.441	0.027	0.006	0.038	0.063	0.100	0.099
Breast	401 NL	0.040	0.207	0.011	0.018	0.060	0.043	0.039
Kidney	944 LL	0.923	0.115	0.198	0.348	0.788	0.750	1.359
Uterus	1138 L	0.173	0.014	0.034	0.038	0.066	0.111	0.073

The mean IC_{50} values were determined for the compound 6 pursuant to the invention on a total of 12 cell lines (Table 3) compared to the therapy standard 5-fluorouracil (5FU) (See Table 2).

A clear superiority of the compound pursuant to the invention over the therapy standard is seen from these figures.

Table 2

Comparison of the antitumoral effect of (6) with the therapy standard 5-fluorouracil (5FU)

Compound	**IC_{50} [µg/ml]**
(6)	0.030
5FU	0.054

Table 3

Tumor cell lines used

Tumor	**Cell line**
Breast	MAXF 401NL
	MCF-7
Colon	HT29
Stomach	GXF251L
Lung	LXFA 629L
	LXFE66L
	LXFL529
Melanoma	MEXF 462NL
	MEXF 514L
Ovary	OVCAR3
Kidney	RXF 944L
Uterus	UXF 1138L

The following examples explain the invention.

Examples

Example 1

Preparation of 2,6-bis(1-aziridino-1-hydroxyiminomethyl)pyridine (6)

Pyridine-2,6-di(carboxamide oxime)

To a solution of hydroxylamine hydrochloride (18.07 g; 26 mmol) and NaOH (10.40 g; 26 mmol) in H_2O (90 ml) is added dropwise with vigorous stirring a solution of pyridine-2,6-dicarbonitrile (12.9 g; 10 mmol) in ethanol (60 ml). An exothermic reaction occurs, and stirring is then continued for 1.5 h at 40-50 °C. After cooling, the precipitate is filtered off and washed with H_2O. Obtained after drying is 16.5 g (85% of the theoretical) of product. M.p. 237-239 °C. ^{1}H NMR. (DMSO-d_6: δ 6.20 (4H, s, NH_2); 7.76 (3H, s, C_5H_3N); 9.76 (2H, s, OH), -CHN (%) found: C 43.6; H 4.5; N 35.9 - calc.: C 43.1; H 4.6; N 35.9.

Pyridine-2,6-dihydroxamic [acid] dichloride

To a cooled solution of pyridine-2,6-di(carboxamide oxime) (1.95 g; 10 mmol) in dilute HCl (20 ml conc. HCl + 8 ml H_2O) is cautiously added dropwise with stirring a solution of $NaNO_2$ (1.78 g; 25 mmol) in H_2O (5 ml). After 1.5 h at 0-10 °C, the solution is stirred for 12 h longer at room temperature. The precipitate is then filtered off and washed with H_2O. Obtained after drying is 2.0 g (79% of the theoretical) of product. M.p. 168-170 °C (dec.), - ^{1}H NMR (DMSO-D_6): δ 8.00 (3H, s, C_5H_3N); 12.7 (2H, s, OH). - CHN (%) found C 33.7; H 2.2; N 16.6 - calc.: C 33.3; H 2.2; N 16.7.

2,6-Bis(1-aziridino-1-hydroxyiminomethyl)pyridine (6)

To a solution of aziridine (0.65 g; 15 mmol) and $N(C_2H_5)_3$ (2.0 g; 20 mmol) in acetonitrile (20 ml) cooled to 0 °C is added dropwise with stirring a suspension of pyridine-2,6-dihydroxamic acid dichloride (1.26 g; 5 mmol) in CH_3CN (20 ml). The mixture is stirred for 90 min and the precipitated triethylamine hydrochloride is filtered off. The filtrate is evaporated under vacuum, and ethyl acetate is added. The mixture is filtered again and the product is washed with $CHCl_3$. Obtained is 0.76 g (60% of the theoretical) of product. M.p. 194-196 °C (dec.). ^{1}H NMR: δ 2.31 (8H, s, CH_2); 7.73 (3H, s, C_5H_3N); 10.64 (2H, s, OH). CHN (%) found: C 52.4; H 5.3; N 27.5 ($C_{11}H_{13}N_5O_2$ x 0.25 H_2O) - calc.: C 52.5; H 5.4; N 27.8.

The following compounds are obtained by an analogous method:

Example 2

1,4-Bis(1-aziridino-1-hydroxyiminomethyl)benzene (7)

M.p. 220-222 °C (dec.). ^{1}H NMR: δ 2.20 (8H, s, CH_2); 7.00 (4H, s, C_6H_4); 12.6 (2H, s, OH). CHN (%) found: C 58.3; H 5.9: N 22.4 ($C_{12}H_{14}N_4O_2$) - calc.: C 58.5; H 5.7; N 22.7.

Example 3

1,4-Di(α-2-carbamoylaziridino-α-hydroxyiminomethyl)benzene (8)

M.p. 248-250 °C (dec.). ^{1}H NMR: δ 2.36 (4H, s, CH_2); 2.82 (2H, m. CH); 7.16 and 7.47 (each 2H, s, s, NH_2); 7.64 (4H, s, C_6H_4); 10.6 (2H, s, OH). CHN (%) found: C 50.3; H 4.9; N 24.9 ($C_{14}H_{16}N_6O_4$) - calc. C 50.6; H 4.8; N 25.3.

Example 4

1,3-Bis(1-aziridino-1-hydroxyiminomethyl)benzene (9)

M.p. 179-181 °C (dec.). ^{1}H NMR: δ 2.17 (8H, s, CH_2); 7.31 (1H, t, C_6H); 7.62 (2H, d, C_6H_2); 8.11 (1H, s, C_6H); 11.3 (2H, s, OH). CHN (%) found: C 58.7; H 5.8; N 22.3 ($C_{12}H_{14}N_4O_2$) - calc.: C 58.5; H 5.7; N 22.7.

Example 5

1,3,5-Tris(1-aziridino-1-hydroxyiminomethyl)benzene (10)

M.p. >300 °C (dec.). ^{1}H NMR: δ 2.16 (12H, s, CH_2); 8.00 (3H, s, C_6H_3); 11.4 (3H, s, OH). CHN (%) found: C 54.1; H 5.4; N 25.0 ($C_{15}H_{18}N_6O_3$) - calc.: C 54.5; H 5.5; N 25.4.

Example 6

1,3-Di(α-2-carbamoylaziridino-α-hydroxyiminomethyl)benzene (11)

M.p. 209-211 °C (dec.). ^{1}H NMR: δ 2.38 (4H, m, CH_2); 3.02 (2H, m, CH); 7.16 and 7.42 (each 2H, s, s, NH_2); 7.42 (1H, t, C_6H); 7.91 (1H, t, C_6H); 10.6 (2H, m, OH). CHN (%) found: C 45.9; H 5.3; N 22.8 ($C_{14}H_{16}N_6O_4$ x 2 H_2O) - calc.: C 45.6; H 5.5; N 22.8.

Example 7

2,6-Di(α-2-carbamoylaziridino-α-hydroxyiminomethyl)pyridine (12)

M.p. 206-208 °C (dec.). ^{1}H NMR: δ 2.38 (4H, m, CH_2); 2.96 (2H, m, CH); 7.11 and 7.40 (each 2H, ss, NH_2); 7.76 (3H, s, C_5H_3N); 10.78 (2H, s, OH). CHN (%) found: C 46.6; H 4.6; N 29.0 ($C_{13}H_{15}N_7O_4$) - calc.: C 46.8; H 4.5; N 29.4.

Example 8

3,5-Bis(1-aziridino-1-hydroxyiminomethyl)pyridine) (13)

M.p. >300 °C (dec.). ^{1}H NMR: δ 2.27 (8H, s, CH_2); 8.29 (1H, t, 4-C_5HN); 8.78 (2H, d, 2,6-C_5H_2N); 11.7 (2H, s, OH). CHN (%) found: C 53.7; H 5.1; N 28.2 ($C_{11}H_{13}N_5O_2$) - calc.: C 53.4; H 5.3; N 28.3.

Example 9

2,5-Bis(1-aziridino-1-hydroxyiminomethyl)pyridine (14)

M.p. 190-192 °C (dec.). ^{1}H NMR: δ 2.22 (4H, s, CH_2); 2.26 (4H, s, CH_2); 7.76 (1H, d, C_5HN); 7.96 (1H, d, C_5HN); 8.78 (1H, s, C_5HN); 11.7 (2H, s, OH). CHN (%) found: C 53.8; H 5.2; N 28.0 ($C_{11}H_{13}N_5O_2$) - calc.: C 53.4; H 5.3; N 28.3.

Example 10

2,4-Bis(1-aziridino-1-hydroxyiminomethyl)pyridine (15)

M.p. >300 °C (dec.). ^{1}H NMR: δ 2.20 (8H, s, CH_2); 7.53 (1H, dd, C_5HN); 8.16 (1H, d, C_5HN); 8.51 (1H, d, C_5HN); 11.6 (1H, s, OH); 11.8 (1H, s, OH). CHN (%) found: C 53.4; H 5.5; N 28.0 ($C_{11}H_{13}N_5O_2$) - calc.: C 53.4; H 5.3; N 28.3.

Example 11

2,5-Bis(1-aziridino-1-hydroxyiminomethyl)furan (16)

M.p. 182-184 °C (dec.). ^{1}H NMR: δ 2.22 (8H, s, CH_2); 6.78 (2H, s, C_4H_2O); 10.5 (2H, s, OH). CHN (%) found: C 47.3; H 5.6; N 22.1 ($C_{10}H_{12}N_4O_4$) - calc.: C 47.2; H 5.6; N 22.0.

Example 12

3,4-Bis[(aziridinyl-1)hydroxyiminomethyl]furoxane (17)

M.p. >300 °C (dec.). ^{1}H NMR: δ 2.18 (4H, s, CH_2); 2.43 (4H, s, CH_2); 11.1 (1H, s, OH); 11.4 (1H, s, OH). CHN (%) found: C 38.2; H 4.2; N 32.9 ($C_8H_{10}N_6O_4$) - calc.: C 37.8; H 4.0; N 33.1.

Example 13

Bis(2-methoxycarbonylaziridino)glyoxime (18)

M.p. 212-214 °C. ^{1}H NMR: δ 2.36 (4H, m, CH_2); 2.96 (2H, m, CH); 3.62 (6H, s, CH_3); 10.71 (2H, s, OH). CHN (%) found: C 42.3; H 5.0; N 19.3 ($C_{10}H_{14}N_4O_6$) - calc.: C 42.0; H 4.9; N 19.6.

Example 14

Bis(2-carbamoylaziridino)glyoxime (19)

M.p. >300 °C. ^{1}H NMR: δ 2.28 (1H, m, CH); 2.40 (1H, m, CH); 2.83 (1H, m, CH); 7.09 and 7.24 (each 1H, s, s, NH_2); 10.65 (1H, s, OH). CHN (%) found: C 37.1; H 4.8; N 32.1 ($C_8H_{12}N_6O_4$) - calc.: C 37.5; H 4.7; N 32.8.

Example 15

2,2'-Azinobis(1-aziridino-1-hydroxyimino)propane (20)

M.p. 172-174 °C. ^{1}H NMR: δ 1.91 (6H, s, CH_3); 2.20 (8H, s, CH_2); 10.9 (2H, s, OH). CHN (%) found: C 46.4; H 4.5; N 32.2 ($C_{10}H_{16}N_6O_2$ x 0.5 H_2O) - calc.: C 46.0; H 6.6; N 32.2.

Example 16

2,2'-Azinobis[1-(2-carbamoylaziridino)-1-hydroxyimino]propane (21)

M.p. 242-244 °C (dec.). ^{1}H NMR: δ 1.98 (6H, s, CH_3); 2.53 (2H, s, CH_2); 2.53 (2H, m, CH_2); 2.89 (2H, m, CH); 7.04 and 7.22 (each 2H, ss, NH_2); 11.02 (2H, s, OH). CHN (%) found: C 41.6; H 5.4; N 32.1 ($C_{12}H_{18}N_8O_4$ x 0.5 H_2O) - calc.: C 41.5; H 5.5; N 32.3.

Example 19 [*sic*]

To test the antiproliferative properties of the compounds pursuant to the invention, a modified propidium iodide assay (Dengler, W.A., Schulte, J., Berger, P.B., Mertelsmann, R., Fiebig, H. H.: Anti-Cancer Drugs 6, 522-532, (1995)) was carried out as described below:

Tumor cells from cell cultures in the exponential growth phase (RPMI Medium, 10% FCS) were harvested, counted, and transferred into 96-well microtiter plates (140 µL cell suspension, 1×10^5 or 5×10^4 cells/mL). After a period of 24 h in which the cells resumed their exponential growth, 10 µL of the test substance dissolved in medium was added to each well (each test concentration was determined in triplicate). After 3-6 days of incubation (depending on the rate of cell doubling), the culture medium was replaced by 200 µL of a fresh medium that contained propidium iodide (25 µg/mL). The microtiter plates were then kept for 24 hours at -18 °C to achieve total cell death. After thawing the plates, fluorescence was measured by means of a Millipore Cytofluor 235 (excitation 530 nm, emission 620 nm). The IC_{50} values of the test compounds were calculated according to the published formula. If an IC_{50} could not be determined within the tested dosage units, the lowest or highest concentration tested was used in each case for the calculation.